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BRYOLOGICAL PATTERNS AND STREAMWATER ACIDIFICATION IN THE VOSGES MOUNTAINS (N.E. FRANCE): AN ANALYSIS TOOL FOR THE SURVEY OF ACIDIFICATION PROCESSES.

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ABSTRACT

The relationships between water chemistry and aquatic bryoflora in the Vosges Mountains (Eastern France) were studied in an attempt to survey the processes of acidification. 6 chemical variables (pH, Mg^{2+}, Ca^{2+}, K^+, NO_3^-, Al) were used to explain the segregation of the bryophytic communities concerning 19 species in 31 study sites. Ca^{2+}, Mg^{2+} and a lesser extend pH and Al are the four variables best explaining the relationships between the bryophytes and the chemical data. Acidophilous species as *Marsupella emarginata* seem to be sensitive to high cations concentrations. On the contrary neutrophilous species as *Rhynchostegium riparioides* may be more sensitive to the toxicity of protons or Al than to lacks of cations. The combinations of bryophytes are used to reconstitute the chemical variables and the precision of the modelling is assessed. ©1998 Elsevier Science Ltd

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INTRODUCTION

Aquatic bryophyte species are often conspicuous elements of the macrophyte vegetation of streams and lakes. In extreme habitats where angiosperms cannot survive, bryophytes may be abundant. Being more shade-tolerant and less sensitive to high hydrostatic pressure, they can occur at rather considerable depths in lakes. Acidic aquatic ecosystems are another extreme habitat in which bryophytes can constitute an important part of the submerged macrophyte vegetation (1, 2, 3).

Studies in Europe and North America have shown that the composition of aquatic bryophytic communities is strongly related to water chemistry. Important interacting factors include acidity (pH), cations concentrations (e.g. Ca^{2+} and K^+) and heavy metals concentrations

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(e.g. Pb and Zn) (4, 5). So far, studies dealing with the distribution of bryophytes related to water quality remain rather scarce. Beside qualitative systems (6), few quantitative analysis have been realised. In the case of acidification problems, the evolution and the ecology of the communities of lenitic systems are much better documented (3, 7, 8, 9, 10, 11) than these of lotic systems. Certain species were combined in a biological key for assessing the water acidification (12, 13).

The Vosges Mountains have been exposed to acidic precipitation since the beginning of the industrialised era. Nevertheless, it is only recently that the existence of acidified streams became apparent (14, 15). In such streams, trout populations and macroinvertebrate communities are severely affected (14, 15, 16, 17).

The aim of this paper is to assess the reaction of the aquatic bryophytic communities according to the water chemistry in the Vosges Mountains (N-E. France). In this paper, we investigate in what extent bryophyte assemblages are linked to the water chemistry and can be used for an easy monitoring of streamwater acidification.

MATERIAL AND METHODS

Study area.

The studied area is located at about 60 km south-west of Strasbourg on the western side of the Vosges Mountains (Donon). Elevations range to 300 m to 1000 m and the climate is temperated oceanic mountainous. The mean annual precipitation is 1500 mm and the mean temperature is 8.4 °C. All the studied streams drain forested catchments (*Abies alba, Picea abies, Fagus sylvatica*) lying on low weathered bedrock (sandstone) and covered by acidic soils ranging from brown acidic soils to podzols (18).

Field and laboratory procedures.

Thirty-one sites scattered along 24 streams were selected in order to provide a wide range of physico- chemical characteristics.

Water samples of 18 sites were recorded monthly from March 1995 to October 1995. 13 additional sites were sampled from September 1995 to March 1996.

pH was measured at the laboratory. Acid Neutralizing Capacity (A.N.C.) was determined by Gran's titrations. Total aluminium concentrations were measured by I.C.P after acidification with HNO_3 and Ca^{++}, Na^+, Mg^{++}, K^+ were measured by atomic absorption spectrometry. Cl^-, SO_4^{--} and NO_3^- were titrated by ion chromatography within 24 hours (Dionex apparatus).

At all sites presence/absence of aquatic or subaquatic bryophytes were recorded. Only submerged or regularly flooded species were included. Hygrophilous species such as *Pellia epiphylla, Brachydontium trichoides* or *Cephalozia bicuspidata*, that are only favoured by the river mesoclimate, were excluded as well as occasionally submerged plants of river banks such as *Sphagnum flexuosum*. Because of the discontinuity and the heterogeneity of aquatic bryophytic habitats, presence of species was preferred to estimation of abundance-dominance. The nomenclature follows the literature for the mosses (19, 20) and for the hepatics (21).

Material of difficult genera such as *Jungermannia* was systematically collected and is deposited at the herbarium of the National Botanic Garden of Belgium.

Descriptive statistics.

The simple statistics of the chemical data were calculated so as to choose the best estimator of each variable. Within the sites, Ca^{2+}, pH, K^{+}, Mg^{2+}, NO_3^- show mostly low coefficients of variation (CV) hence the use of the mean as an estimator of these variables. On the contrary Al show very often important CV within the sites; the mean of Al is consequently not a precise indicator of Al, hence the use of the interval of confidence (IC) of the mean calculated for alpha = 0.05. As Al can be considered as a potential toxic element for the bryoflora, only the upper value of the IC was considered. So as to choose the best model to study the relationships between the bryophytes and the chemical data, the shape of the species response curve was drawn by dividing the chemical variable into classes and calculating the frequency of occurrence for each class (22). As the number of samples n_i per class K_i was not constant, the number of occurrences of each species in K_i was divided by n_i. The response curves of species, often more or less roughly linear, suggested to use linear models for this study. The bryophytic data were arranged by Principal Components Analysis and classified by Twinspan, a divise method of classification ordering the samples according to the species by using the centroïd of the first axis of an ordination as the limit between two groups (23). The assessment of the relation between the bryophytes and the chemical variables was realised by Canonical Correlation Analysis and Canonical Redundancy Analysis performed with SAS. Can Corr is the procedure calculaing from the matrix including both sets of data (bryophytes and chemical data in each site) linear combinations of bryophytes - called canonical variable for the bryophytes - and linear combinations of chemical variables - called canonical variable for the chemical data - so that the correlation between both canonical variables is the highest. The number of repetitions of this procedure equals the smallest number of variables of the smallest set of variables, i.e. the six chemical variables in this study. The Redundancy Analysis examinates in what extent the initial variables can be estimated from the canonical variables. For the p chemical variables c, the standardized variance explained by their own canonical variables C is: $1/p \times \sum^{p}_{i=1} r^2 (c, C)$ and the standardized variance explained by the opposite canonical variable B is: $(1/p \times \sum^{p}_{i=1} r^2 (c, C) \times r^2 (C, B)$. The percentage variance of the chemical data explained by the first canonical variable of the bryophytes is obtained by multiplying the first canonical coefficient of correlation by the percentage variance of the chemical data explained by their own canonical variable. This is an average percentage variance and certain variables well correlated with the canonical variable are better reconstituted than those variables which are less correlated with the canonical variable. The chemical variables best correlated with the canonical bryophytic variables were reconstituted from the bryological canonical variables by least-squares regression.

RESULTS

Physico-chemical characteristics.

Table 1 summarises the values of the main chemical variables for each sampling site.

N° sites	pH	ANC µeq/l	total Al µg/l	Ca^{2+} mg/l	K$^+$ mg/l	Mg^{2+} mg/l	Na$^+$ mg/l	Cl$^-$ mg/l	N-NO$_3^-$ mg/l	SO$_4^{2-}$ mg/l
D 1	4.33	-46	1057	0.87	0.83	0.35	0.85	1.45	1.29	5.76
D 2	4.44	-37	849	1.41	1.03	0.54	1.03	1.69	1.45	6.26
D 3	4.47	-45	1239	0.80	0.73	0.29	0.78	1.15	1.19	6.40
D 4	4.48	-25	1029	1.02	1,00	0.43	0.95	1.39	1.20	5.87
D 5	5.02	8	706	1.51	1.68	0.63	0.92	1.89	0.17	7.41
D 6	5.14	-6	241	2.00	1.57	0.89	0.91	3.10	0.62	8.25
D 7	5.17	-1	255	2.40	1.66	1.04	0.95	2.09	0.91	9.46
D 8	5.26	-6	216	1.96	1.70	0.96	0.94	1.81	0.58	9.04
D 9	5.27	16	378	1.77	1.53	0.70	0.92	1.39	1.22	5.02
D 10	5.40	31	133	1.85	1.65	0.71	1.15	1.52	0.77	5.53
D 11	5.45	3	128	2.86	1.81	1.20	1.15	2.36	0.83	10.78
D 12	5.62	10	63	2.96	1.84	1.16	1.61	3.19	0.64	10.75
D 13	5.68	12	113	3.01	1.67	1.15	1.01	2.02	1.01	9.96
D 14	5.69	5	62	2.35	1.98	1.18	1.17	2.20	0.36	11.13
D 15	5.69	7	174	2.29	1.61	0.91	0.89	1.88	0.59	8.55
D 16	5.69	15	58	2.84	1.85	1.10	1.15	1.98	0.80	9.99
D 17	5.85	36	29	2.37	1.78	0.96	1.40	2.01	0.85	7.24
D 18	5.87	15	60	2.89	1.79	1.15	1.16	2.08	0.65	9.68
D 19	5.90	31	318	2.39	1.34	0.93	0.79	1.33	0.56	6.03
D 20	5.90	17	55	2.97	1.82	1.14	1.42	2.06	0.65	10.18
D 21	5.93	39	50	2.21	1.73	1.03	1.38	2.08	0.67	7.56
D 22	6.00	20	32	2.89	2.01	0.91	1.14	1.90	0.61	9.64
D 23	6.18	37	105	3.31	2.46	1.13	5.64	10.09	0.61	7.62
D 24	6.46	188	95	3.97	1.55	1.96	1.25	1.75	0.95	7.19
D 25	6.77	624	19	8.38	1.75	4.25	1.13	1.63	0.85	6.27
D 26	6.83	278	113	5.30	1.59	2.53	0.86	1.38	0.61	5.70
D 27	6.88	168	77	4.25	2.06	1.81	3.25	6.61	0.20	7.46
D 28	6.91	129	51	3.26	1.33	1.53	1.03	1.40	0.97	6.32
D 29	7.07	839	18	12.68	1.46	4.43	1.18	1.63	0.96	6.29
D 30	7.17	219	45	4.53	1.61	2.03	3.71	6.22	1.06	6.94
D 31	7.32	746	72	11.55	1.33	4.18	1.3	1.73	0.91	7.28

Table 1: Physico-chemical characteristics of sampling sites (mean values).

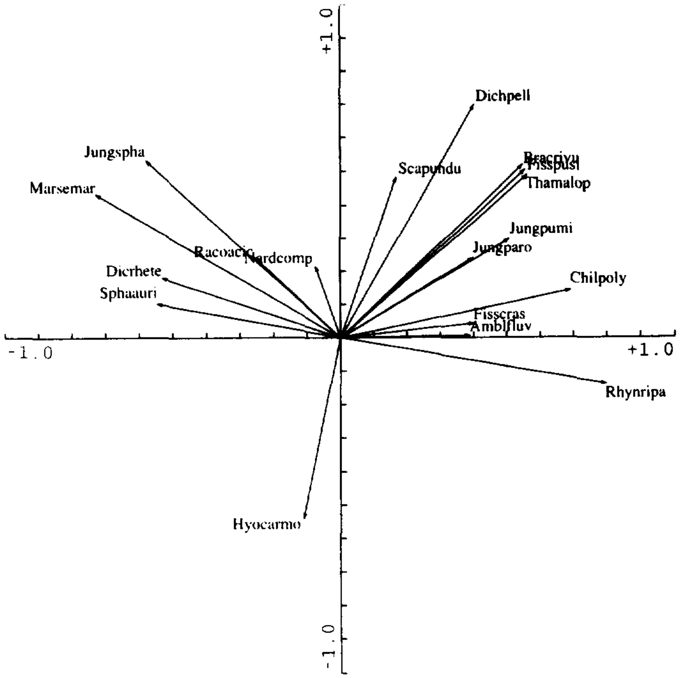
Water of sampling sites ranges from strongly acidified to neutral. The most acidic streams are characterized by a mean pH value ≤ 4.5 (*i.e.* [H$^+$] ≥ 32µeq/l), a mean total [Al] ≥ 850µg/l and a mean Ca concentration ≤ 1.4mg/l (*i.e.* [Ca^{++}] ≤ 70µeq/l).

At the opposite the water of the neutral streams have a mean pH = 7.0 (*i.e.* [H$^+$] < 1µeq/l), a mean total Al concentration ≤ 75µg/l and a mean [Ca] ≥ 4.5mg/l (*i.e.* [Ca^{++}] ≥ 226µeq/l).

The physico-chemical survey of the degree of soft-water acidification can be use for predicting the effects of water acidification on aquatic bryophyte communities.

Ecological characterisation of the bryophytes.

The P.C.A. realised from the data matrix species x sites, shows a physico-chemical gradient along the first axis having an eigenvalue of 0.28, with species typical of acidic waters with a very low buffer capacity such as *Hyocomium armoricum* and *Racomitrium aciculare* on the left and species typical of acidic to neutral waters such as *Chiloscyphus polyanthos* and *Rhynchostegium riparioides* on the right.



Axis 1: acidification gradient (right to left)

Figure 1: Principal components analysis of the data matrix species x sites.

The constrained analysis realised on the data matrix species-chemical data x sites precises the ecological gradient suggested by the PCA, *i.e.* the relationships between the environmental data and the bryoflora. The first axis of the Canonical Correlation Analysis explains 93% of the relation between the bryophytes and the chemical variables and 12% of the variance of the bryophytes (Table 2).

	Axis 1		Axis 2	
Canonical correlation	0.998***		0.965*	
Eigenvalue	360.749		13.473	
Proportion	0.927		0.034	
	$r^2_{b.c.}$	c. coeff.	$r^2_{b.c.}$	c. coeff.
Sphagnum auriculatum	-0.348	-0.233	-0.044	0.404
Scapania undulata	-0.281	-0.266	-0.619	-0.752
Nardia compressa	-0.041	-0.097	0,106	-0.106
Marsupella emarginata	-0.312	-0.198	-0.286	-0.410
Jungermannia sphaerocarpa	-0.130	0.229	-0.294	-0.417
Jungermannia plectocolea	0.284	-0.266	0.066	-0.04
Jungermannia pumila	0.319	0.125	-0.005	0.178
Hyocomium armoricum	-0.026	0.234	-0.153	-0.211
Rhynchostegium riparioides	0.349	-0.327	0.360	0.169
Chiloscyphus polyanthos	0.534	0.191	0.180	-0.434
Brachythecium rivulare	0.319	-0.296	0.183	0.818
Dicranella heteromalla	-0.101	-0.059	-0.285	0.175
Fissidens pusillus	0.573	0.570	0.197	0.096
Fissidens crassipes	0.507	0.389	-0.089	-0.330
Racomitrium aciculare	-0.112	-0.083	0.108	0.403
Dichodontium pellucidum	0.498	0.221	0.124	0.340
Amblystegium fluviatile	0.026	-0.608	0.212	0.478
Thamnobryum alopecurum	0.441	0.718	0.072	-1.039
Rhizomnium punctatum	0.453	0.010	0.130	0.122
$r^2_{c.b.}$				
Ca	0.929		0.268	
K	-0.150		0.382	
pH	0.511		0.551	
$N\text{-}NO_3^-$	0.178		-0.261	
Al	-0.120		-0.626	
Mg	0.872		0.454	
% Var. b.c.	11.9		5.3	
% Var. c.b.	32.6		19.9	

Table 2: Summary of the Canonical Correlation Analysis and the Canonical Redundancy Analysis

***** : very highly significant**

*** : significant**

$r^2_{b.c}$: correlation between the bryophytes and the opposite canonical variable

c. coeff. : standardized coefficients of the bryophytes

$r^2_{c.b.}$: correlation between the chemical variables and the opposite canonical variable

% Var. b.c. : percentage variance of the bryophytes explained by the opposite canonical variable

% Var. c.b. : percentage variance of the chemical variables explained by the opposite canonical variable.

This axis is highly correlated with Ca, Mg and in a lesser extent with pH. The most correlated bryophytes with this axis are *Chiloscyphus polyanthos, Fissidens pusillus, Fissidens crassipes, Dichodontium pellucidum, Rhizomnium punctatum, Thamnobryum alopecurum*, which can be all considered as neutrophilous species.

The second axis of the Canonical Correlation Analysis explains 3.5% of the relations between the bryophytes and the chemical variables and 5.5% of the variance of the bryophytes. This axis is mostly correlated with Al and pH and with *Scapania undulata, Marsupella emarginata, Jungermannia sphaerocarpa, Rhynchostegium riparioides* and *Dicranella heteromalla*, which are mostly acidophilous species.

To precise the response of the species according to the variations of the physico-chemical parameters best correlated with the two first axis, conditional frequencies - *i.e.* the relative frequencies of the species within the classes of environmental data - were calculated and are shown in Table 3.

species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Al(μg/l)																			
< 100	0.31	0.85	0.00	0.31	0.15	0.00	0.08	0.38	0.69	0.31	0.15	0.08	0.23	0.15	0.08	0.23	0.08	0.08	0.23
100-250	0.00	0.83	0.17	0.17	0.17	0.33	0.17	0.67	0.83	0.33	0.33	0.00	0.50	0.00	0.17	0.33	0.00	0.17	0.33
250-500	0.67	1.00	0.33	0.67	0.33	0.17	0.00	1.00	0.17	0.33	0.17	0.00	0.00	0.00	0.33	0.17	0.00	0.00	0.33
500-1000	0.33	1.00	0.00	1.00	0.67	0.00	0.00	1.00	0.00	0.00	0.00	0.67	0.00	0.00	0.33	0.00	0.00	0.00	0.00
> 1000	0.33	1.00	0.00	1.00	1.00	0.00	0.00	0.67	0.00	0.00	0.00	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Ca^{2+} (mg/l)																			
< 1.5	0.00	1.00	0.00	1.00	0.75	0.00	0.00	0.75	0.00	0.00	0.00	0.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1.5-2.5	0.27	1.00	0.18	0.73	0.45	0.09	0.09	0.73	0.18	0.18	0.09	0.18	0.00	0.00	0.27	0.09	0.00	0.00	0.27
2.5-3	0.00	0.83	0.00	0.17	0.00	0.00	0.00	0.67	0.83	0.00	0.00	0.00	0.17	0.00	0.00	0.00	0.00	0.00	0,00
3-5	0.17	0.83	0.00	0.17	0.17	0.17	0.00	0.33	0.67	0.50	0.33	0.00	0.33	0.17	0.33	0.33	0.17	0.17	0.17
> 5	0.75	0.75	0.25	0.25	0.25	0.25	0.25	0.75	1.00	0.75	0.50	0.00	0.75	0.25	0.00	0.75	0,00	0.25	0.75
Mg^{2+}(mg/l)																			
< 0.5	0.33	1.00	0.00	1.00	1.00	0.00	0.00	0.67	0.00	0.00	0.00	0.33	0.00	0.00	0,00	0,00	0.00	0.00	0.00
0.5-1	0.50	1.00	0.20	0.70	0.50	0.10	0.00	0.90	0.20	0.10	0.10	0.30	0.00	0.00	0.20	0.10	0.00	0.00	0.20
1-1.5	0.30	0.80	0,00	0.30	0.00	0.00	0.10	0.50	0.60	0.20	0.00	0.00	0.10	0,00	0.10	0,00	0,00	0,00	0.20
1.5-4	0.20	1.00	0.20	0.40	0.40	0.20	0,00	0.40	0.80	0.40	0.60	0.00	0.60	0.20	0.40	0.60	0.20	0.20	0.00
> 4	0.00	0.67	0.00	0.00	0.00	0.33	0.33	0.67	1.00	1.00	0.33	0.00	0.67	0.33	0.00	0.67	0,00	0.33	1.00
pH																			
< 5	0.33	1.33	0.00	1.33	1.00	0.00	0.00	1.00	0.00	0.00	0.00	0.67	0.00	0.00	0.00	0.00	0.00	0.00	0.00
5-5.5	0.50	0.70	0.10	0.50	0.20	0.00	0.00	0.70	0.10	0.10	0.00	0.10	0.00	0.00	0.20	0.00	0.00	0.00	0.10
5.5-6	0.30	0.80	0.10	0.40	0.30	0.10	0.10	0.40	0.50	0.10	0.10	0.10	0.10	0.00	0.10	0.10	0.00	0.00	0.20
6-7	0.20	1.20	0.20	0.40	0.40	0.20	0.00	1.00	1.20	0.60	0.40	0.00	0.40	0.20	0.40	0.40	0.00	0.00	0.40
> 7	0.00	1.00	0.00	0.00	0.00	0.33	0.33	0.33	1.00	1.00	0.67	0.00	1.00	0.33	0.00	1.00	0.33	0.67	0.67

1: S. auriculatum; 2: S. undulata; 3: N. compressa; 4: M. emarginata; 5:J. sphaeocarpa; 6: J. plectocolea; 7:J. pumila; 8: H. armoricum; 9:R. riparioides;10:C. polyanthos; 11: B. rivulare; 12:D. heteromella; 13: F. pusillus; 14: F. crassipes; 15: R. aciculare; 16: D. pellucidum; 17:A. fluviatile; 18: T. alopecurum; 19: R. punctatum.

Table 3: Conditional frequencies of the species according to the physico-chemical data.

Sphagnum auriculatum is the only species showing an optimum at Mg average concentrations of 0.50-1.00 mg/l, pH of 5-5.5, and disappears in waters with [Mg] superior to 4.00mg/l and pH superior to 7.0. Other species show often more or less linear response curves. Certain species prove to be tolerant to high Al concentrations: *Scapania undulata, Marsupella emarginata* and *Jungermannia sphaerocarpa*, and are especially concerned, having a relative frequency of 1 for Al maxima concentrations superior to 1000μg/l. Some species decrease with increasing cations concentrations and pH. This is namely the case for *Jungermannia sphaerocarpa* and *Marsupella emarginata*, disappearing when Mg average concentrations become superior to 4.00 and if the pH reaches 7.0. Another acidophilous species, *Dicranella heteromalla*, even disappears when Ca and Mg average concentrations and pH respectively become superior to 2.50, 1 and 6.0.

On the contrary, some other species increase with increasing cations concentrations and pH, e.g. *Chiloscyphus polyanthos*, only present when Mg average concentrations are superior to 0.50 and pH to 5.5, *Fissidens pusillus*, that needs Ca and Mg average concentrations respectively superior to 1.50 and 1.00mg/l and a pH superior to 5.5, and lastly *Thamnobryum alopecurum*, growing in waters with Ca and Mg average concentrations respectively superior to 3.00 and 1.50mg/l and a pH superior to 7.0.

Reconstitution of the chemical variables from the bryological data

Once having defined the species ecology, the bryophytic assemblages can be used in a typology of waters.

	first group					second group			third group								fourth group								fifth group								
sites	D1	D4	D2	D9	D17	D28	D5	D3	D10	D21	D7	D14	D8	D6	D12	D13	D24	D19	D26	D20	D11	D22	D18	D16	D30	D31	D29	D15	D23	D25	D27	**Mean**	**Std Dev**
D. heteromella	1	1	1	1	1																											0.13	0.34
M. emarginata	1	1	1	1	1	1	1	1	1	1	1		1		1	1		1	1													0.52	0.51
J. sphaerocarpa	1	1		1	1	1	1	1										1	1									1				0.32	0.48
S. auriculatum					1	1	1	1	1		1	1	1	1	1	1																0.35	0.49
R. aciculare				1		1					1						1	1														0.16	0.37
R. riparioides										1					1	1	1	1	1	1	1	1	1	1	1	1	1		1	1	1	0.55	0.51
N. compressa														1				1	1													0.10	0.30
F. pusillus															1		1		1						1	1	1					0.19	0.40
J. plectocolea																	1									1		1				0.10	0.30
J. pumila										1																1	1					0.06	0.25
C. polyantnos											1					1									1	1	1	1	1	1	1	0.29	0.46
R. punctatum										1			1			1										1	1	1	1	1		0.26	0.44
B. rivulare																			1						1	1		1			1	0.16	0.37
F. crassipes																											1				1	0.06	0.25
A. fluviatile																									1					1		0.03	0.18
T. alopecurum																									1	1						0.06	0.25
D. pellucidum						1										1		1	1						1	1	1					0.23	0.43
H. armoricum		1	1	1			1	1	1		1		1	1		1	1	1	1	1	1	1	1	1			1	1	1	1		0.71	0.46
S. undulata	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1		1	0.97	0.18
Al (µg/l)	428-798					458-881			114-177								90-165								49-122								
Ca^{++} (mg/l)	1.30-1.80					1.40-2.30			2.30-2.50								3.00-3.60								4.2-8.5								
Mg^{++} (mg/l)	0.53-0.71					0.56-1.05			1.00-1.09								1.22-1.56								1.64-3.38								
pH	4.6-5.2					4.9-5.8			5.3-5.5								5.8-6.1								6.3-6.9								

Table 4: Ordered classification of the records according to the bryophytes.

The results of the ordered classification of the records according to the bryophytes, stopped at the third partitioning level, show 5 groups of sites characterised by their floristic combinations and chemical values (Table 4).

- a first group, concerning strongly acidic streams characterised by a pH between 4.6 and 5.2 and Ca between 1.30 and 1.80mg/l, including *Marsupella emarginata, Jungermannia sphaerocarpa* and *Dicranella heteromalla*;

- a second group with a pH of 4.9-5.8 and Ca of 1.40-2.30mg/l differentiated from the former by the absence of *Dicranella heteromalla* and the presence of *Sphagnum auriculatum*;

- a third group with a pH of 5.3-5.5 and Ca between 2.30 and 2.50mg/l differentiated by the occasional presence of neutrophilous species such as *Rhynchostegium riparioides* and *Chiloscyphus polyanthos* ;

- a fourth group with a pH of 5.8-6.1 and Ca of 3.00-3.60mg/l characterised by the absence of *Sphagnum auriculatum*, the rarety of *Marsupella emarginata* and the presence of the neutrophilous mosses including *Rhynchostegium riparioides* and sometimes *Fissidens pusillus* and *Dichodontium pellucidum*;

- a fifth group having a pH of 6.3-6.9 and Ca of 4.20-8.50mg/l characterised by the absence of the acidophilous species such as *Marsupella emarginata* and the presence of *Fissidens pusillus, Dichodontium pellucidum, Chiloscyphus polyanthos,* sometimes *Thamnobryum alopecurum*.

More precisely, the chemical variables best correlated with the bryophytes can be reconstituted by calculation. The first bryological canonical variable explains 32.6% of the variance of the chemical data (Table 2). Ca and Mg and in lesser extend with pH are the best reconstituted variables on this axis. The second bryological canonical variable explains 19.89% of the variance of the chemical variables, of which Al and pH are the best reconstituted. It is now possible to reconstitute the chemical variables by least-squares regression between the chemical variables and the bryophytic canonical variables (Table 5).

Dependent variable	**Ca^{2+}**	**Mg^{2+}**	**pH**	**total Al**
Explaining variable	Axis 1	Axis 1	Axis 2	Axis 2
r^2 adj	0.860	0.753	0.284	0.371
prob.>F	0.0001	0.0001	0.0012	0.0002
coefficient	2.558	0.941	0.474	-242.318
standard error	0.188	0.097	0.132	55.992
constant	3.382	1.393	5.829	322.819
standard error	0.185	0.096	0.129	55.081

Table 5: Summary of the regressions between the chemical variables and the bryophyte canonical variables.

One can now calculate the chemical parameters of a new site by calculating the bryophytic canonical variable- i.e. by multiplying the standardised value of each bryophyte by its standardised coefficient- and using it in the equation of regression. The quality of the regression is assessed by the adjusted coefficient of determination and the standard error of the estimated parameters.

DISCUSSION

Ecological and ecophysiological effects of low pH and associated ions concentrations.

The gradients of acidity were already designated as the cause of the segregation of the aquatic bryoflora. In the streams of 5 German siliceous mountain areas, aquatic bryophyte vegetation show a remarkable segregation along a pH gradient (12, 13). For example, when the pH of waters was below 5.0 and the buffer capacity below 100µeq/l, species such as *Chiloscyphus polyanthos* were excluded whereas the acid-indicating species group of *Scapania undulata* was mainly existent in these areas. This can be explained physiologically by two main ecophysiological effects of a low pH (*i.e.* high H^+ concentration) on aquatic bryophytes.

A first group of effects is linked to the increase of certain toxic elements. *Scapania undulata* tolerates high levels of Al in its environment (24). This is corroborated in the Vosges by the resistance of certain species such as *Scapania undulata*, *Jungermannia sphaerocarpa* and *Marsupella emarginata* to high Al concentrations. Nevertheless Al was not a major element explaining the relationships between the bryoflora and the chemical data. Effects of Al and other toxic metal ions are complicated by other factors, including organic matter chelation and antagonistic effects of cations such as Ca^{2+} (25, 26). It has been shown that competition between Al and Ca^{2+} influx may bring about immediate changes in the cell Ca^{2+} homeostasis, while as the length of time of contact with Al increases, an absence (or reduction) of net Ca^{2+} influx at the root tip may cause localised Ca deficiency, affecting both structure and function of membranes and cells (27). In the Vosges, it is shown that the cation concentrations - although unmeasured correlated variables cannot be excluded - and the pH that best explain the relationships between the bryoflora and the physico-chemical parameters. The presence of species known in the literature as calcicolous such as *Rhynchostegium riparioides* in waters with very low Ca concentrations (as low as 2.00mg/l) but with a pH always superior to 5.0, or such as *Thamnobryum alopecurum*, that appears in waters with Ca superior to 3.00mg/l and Mg to 1.50mg/l, was unexpected. In the Vosges mountains, these species were more neutrophilous than calcicolous. The ecology of such species could be more linked to protons and/or Al concentrations than to the high concentrations of cations. Effects of protons on the development of the protonema were already demonstrated, being more important for *Rhynchostegium riparioides* and *Chiloscyphus polyanthos* and a bit less for *Scapania undulata* (12, 13). In Japan, *Rhynchostegium riparioides* was even found in weakly acidified (pH 6.1) to neutral (pH 6.7) streams (28). These effects are partially due the competition between protons and the pool of cations absorbed by the plants. Low pH cause the cations to be displaced from exchange sites, thus causing cations in the cell to bind to the walls and deplete the cell's cation content (29). Such species may perhaps be better qualified of sensitive to protons and aluminium. Similar results were obtained in a comparative study of two waterfalls in the Eastern Vosges; the water of the first waterfall, despite rather low concentrations of e.g. Ca of 6.00mg/l, had a pH of ca. 7.5 due to the very low concentration of CO_2 at the base of the waterfall. The presence of species such as *Rhynchostegium riparioides* or *Thamnobryum alopecurum* allowed to think that these species are not linked to high cations concentrations but that their development is inhibited by the high H^+ concentrations. On the other hand, acidophilous species such as *Marsupella emarginata* or *Racomitrium aciculare* are not inhibited by high pH but by high cations concentrations, which was emphasized by their absence in the second waterfall, with the same pH but with a higher conductivity (30). Species such as *Marsupella emarginata* and *Scapania undulata*, typically qualified of acidophilous, seem to possess a physiological system regulating the difference in H^+ concentrations between outside (acidic water) and inside (protoplast) to maintain the activity for growth in acidic environments (28). Adapted to low pH, they can also occur in waters with pH close to 7.0 but decrease in waters with Ca concentrations that are not very high; in this case, the predominance of one or some cations is thus a limiting factor probably linked to the fact that such cations limitate the penetration of other elements needed by the plants within the cells. These plants would not be physiologically adapted to evacuate the excedent of cations from the walls of cell. These cations could be more important than the pH of waters for such species, which may be present in waters with a high pH but with low cations concentrations, so that they would not be real acidophilous species but rather sensitive to cations.

A second group of effects is linked to troubles in the plants nutrition, in particular the NH_4^+/NO_3^- ratio which favouring taxa such as the *Sphagnum* (31) in acidic conditions. Nevertheless, the studies on the influencing of carbon dioxide and ammonium in a culture experiment on the growth of *Sphagnum cuspidatum*, have shown that ammonium enrichment without carbon dioxide enrichment does not lead to an increase in biomass of *S. cuspidatum* (32). In fact N was not detected as an influent element in this analysis. Ammonium concentrations seem to be less important than carbon dioxide concentration which is of vital importance for the growth of submerged macrophytes. Low pH cause all CO_2 to be present as free CO_2, making it possible for the bryophytes to obtain adequate CO_2 for photosynthesis (33) hence the presence in streams of usually terrestrial taxa (34). This result is corroborated in the Vosges, with the presence of the forest moss *Dicranella heteromalla* in the most acidic streams nearby *Marsupella emarginata* and *Jungermannia sphaerocarpa*.

Assessment of the possibilities to use aquatic bryophytes for surveying the acidification of the streams.

The system of bioindication based on the organisation of a phytosociological table proposed for the Vosges is rather similar to the qualitative system showing that *Scapania undulata, Marsupella emarginata, Jungermannia sphaerocarpa* and sometimes *Hyocomium armoricum, Warnstorfia fluitans* are present in permanently strongly acidified waters (12, 13). *Rhynchostegium riparioides* and perhaps *Hygrohypnum ochraceum* colonise neutral or periodically acidified streams, whereas *Chiloscyphus polyanthos, Brachythecium rivulare, Fontinalis antipyretica* and sometimes *Fontinalis squamosa, Brachythecium plumosum,* *Riccardia chamaedryfolia, Fissidens crassipes, Amblystegium tenax* characterise neutral to periodically slightly acidified streams.

Nevertheless, this kind of floristic classification leads obviously in the Vosges to missclassify some sites, *e.g.* D17 and D28, that are classified in the acidic streams according to the floristic method but which are moderately and slightly acidified respectively according to the chemical data. This as well true for the sites D24 and D25 which appear on the extreme right of the floristic classification but which belong to the category of slightly to neutral acidic streams according to the chemical data. It is possible that the number of chemical measures was too low to integrate the whole variability of these streams, where episodic stresses may have happened. But it must be emphasized that all the bryophytes have not the same biological value. For example, the only presence of a single moss characteristic of neutral streams such as *Dichodontium pellucidum*, has not enough influence on the classification to rectify the position of a site as D28 closer to sites such as D19. Moreover, in the case of original ecological conditions such as the waterfalls, different ecological bryophyte communities can be present, and the water chemistry is thus very difficult to assess with the classical methods of floristic clusters (30). Within this framework, a canonical analysis could be a more precise tool because of affecting a different weight to each species in relation to its correlation with the chemical variables. This approach enables moreover the assessment of the quality of the reconstitution of the chemical parameters by giving an interval of confidence. Such methods could thus become a rather performant tool for the survey of the water quality in a defined area. Nevertheless these systems can probably not be used in other phytogeographical districts, where the competition within the plants communities and even the ecotypes of a taxon can be different - the case of *Jungermannia sphaerocarpa*, qualified as calcicolous (35) but definitely inhibited by high calcium concentrations in the Vosges, in Belgium and in Germany, being an example of this.

CONCLUSION

This work shows the tendencies of the bryophytic flora to be distributed first according to a gradient of mineralization (calcium) and second according to a gradient of acidification (pH, aluminium). Acidophilous species seem to be sensitive to high cations concentrations, whereas neutrophilous species may be more sensitive to the toxicity of protons or aluminium than to lacks of cations, but interactions between ions toxicity and nutrition troubles occur. Within this framework, further studies about the ecophysiological and cytological effects of toxic elements such as aluminium and protons should be realised. The capacity of species to tolerate nutrition stresses should be measured in experimental laboratory conditions. Nevertheless, one of the most important problems of bioindication, i.e. the total lack of standardisation of the measures and methods, remains entirely.

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